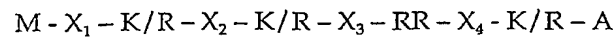


CLAIMS

1. A recombinant polynucleotide, the polynucleotide comprising a first and a second sequence, the first sequence encoding a signal peptide comprising a TAT signal and a Sec avoidance signal and the second sequence encoding a heterologous protein,
 5 wherein the sequence of the signal peptide is



in which X_1 is a sequence of 0 to 10 amino acids;

X_2 is a sequence of 0 to 3 amino acids;

X_3 is a sequence of 0 to 10 amino acids; and

- 10 X_4 is a sequence of 15 to 24 amino acids in which at least 75% up to about 90% of the residues are hydrophobic.

2. A recombinant polynucleotide according to claim 1 wherein X_1 is a sequence of 0 to 5 amino acids, and is preferably 0.

3. A recombinant polynucleotide according to claim 1 or 2 wherein X_2 is a
 15 sequence of 0 or 1 amino acid, preferably 0.

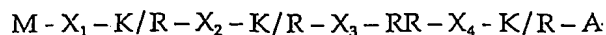
4. A recombinant polynucleotide according to any one of claims 1 to 3 wherein X_3 is a sequence of 0 to 5 amino acids, preferably 0.

5. A recombinant polynucleotide according to any one of claims 1 to 4 wherein X_4 is a sequence of at least 20 amino acids of which at least 18 are hydrophobic amino
 20 acids.

6. A recombinant polynucleotide according to any one of claims 1 to 5 wherein X_4 is 23 amino acids.

7. A recombinant polynucleotide according to any one of claims 1 to 6 wherein the sequence of the signal peptide is MKKRRVNSVLLLLLLASALALTVPMAKA
 25 (SEQ ID NO: 1).

8. A signal peptide, the signal peptide having the sequence



in which X_1 is a sequence of 0 to 10 amino acids;

X_2 is a sequence of 0 to 3 amino acids;

X_3 is a sequence of 0 to 10 amino acids; and

5 X_4 is a sequence of 15 to 24 amino acids in which at least 75% up to about 90% of the residues are hydrophobic.

9. A signal peptide according to claim 8 wherein X_1 is a sequence of 0 to 5 amino acids, and is preferably 0.

10. A signal peptide according to claim 8 or 9 wherein X_2 is a sequence of 0 or 1 amino acid, preferably 0.

11. A signal peptide according to any one of claims 8 to 10 wherein X_3 is a sequence of 0 to 5 amino acids, preferably 0.

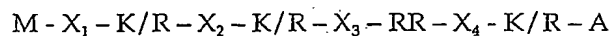
12. A signal peptide according to any one of claims 8 to 11 wherein X_4 is a sequence of at least 20 amino acids of which at least 18 are hydrophobic amino acids.

15 13. A signal peptide according to any one of claims 8 to 12 wherein X_4 is 23 amino acids.

14. A signal peptide according to any one of claims 8 to 13 wherein the sequence of the signal peptide is MKKRRVNSVLLLLLLASALALTVPMAKA (SEQ ID NO 1).

20 15. A method of producing a heterologous polypeptide from a host cell comprising a TAT translocation system, the method comprising:

(i) transforming the host cell with a DNA sequence encoding the heterologous polypeptide and a signal peptide wherein the signal peptide comprises a TAT signal and a Sec avoidance signal wherein the sequence of the signal peptide is



25 in which X_1 is a sequence of 0 to 10 amino acids;

X₂ is a sequence of 0 to 3 amino acids;

X₃ is a sequence of 0 to 10 amino acids; and

X₄ is a sequence of 15 to 24 amino acids, in which at least 75% up to about 90% of the residues are hydrophobic.

- 5 (ii) culturing the host cell under conditions which allow expression of the heterologous polypeptide; and
- (iii) recovering the heterologous polypeptide secreted from the host cell via the TAT translocation system.

10 16. A method according to claim 15 wherein X₁ is a sequence of 0 to 5 amino acids, and is preferably 0.

17. A method according to claim 15 or 16 wherein X₂ is a sequence of 0 or 1 amino acid, preferably 0.

18. A method according to any one of claims 15 to 17 wherein X₃ is a sequence of 0 to 5 amino acids, preferably 0.

15 19. A method according to any one of claims 15 to 18 wherein X₄ is a sequence of at least 20 amino acids of which at least 18 are hydrophobic amino acids.

20. A method according to any one of claims 15 to 19 wherein X₄ is 23 amino acids.

20 21. A method according to any one of claims 15 to 20 wherein the sequence of the signal peptide is MKKRRVVNSVLLLLLLASALALTVPMAKA (SEQ ID NO: 1).

22. A method according to any one of claims 15 to 21 wherein the host cell is *Bacillus sp.*

25 23. A method according to claim 22 wherein the host cell is selected from the group consisting of *Bacillus choshinensis*, *Bacillus brevis*, *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus megaterium*.

24. A method according to claim 22 wherein the host cell is *Bacillus choshinensis*.

25. A method according to any one of claims 15 to 24 wherein the heterologous polypeptide is a polypeptide which readily folds in the cytoplasm.
26. A method according to any one of claims 15 to 25 wherein the polynucleotide encoding the mature polypeptide has a sequence selected from:
- 5 (i) a sequence of nucleotides shown in SEQ ID NO:29 from nucleotide 85 to 1155;
- (ii) a sequence that hybridises to SEQ ID NO:29 from nucleotide 85 to 1155 under conditions of high stringency;
- 10 (iii) a sequence which is greater than 90% identical to SEQ ID NO:29 from nucleotide 85 to 1155; and
- (iv) a sequence that encodes the amino acid sequence provided in SEQ ID NO:30 from residue 29 to 384.
27. A method according to any one of claims 15 to 25 wherein the mature heterologous polypeptide comprises the sequence provided in SEQ ID NO:30 from
15 residue 29 to 384; or a polypeptide which is greater than 90% identical to the sequence provided in SEQ ID NO:30.
28. A substantially purified polypeptide produced according to the method of any one of claims 15 to 27.
29. A vector comprising the recombinant polynucleotide according to any of
20 claims 1 to 8.
30. A host cell comprising the recombinant polynucleotide according to any of claims 1 to 8.